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CRITERIA FOR THE IDENTIFICATION OF THE MORE COMMON INTESTINAL PARASITES, WITH METHODS FOR THE PREPARATION AND EXAMINATION OF STOOLS*

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Numerous reviews and surveys from all parts of the country have shown everywhere a high incidence of intestinal parasites and have served to dispel the idea that their incidence is of importance only in certain areas and more particularly in the South. The predominating type of parasite is not the same in all reports, it is true, but the incidence is usually relatively high, regardless of location. The ability, therefore, to recognize the various types of intestinal parasites which are more commonly encountered, as well as to recognize as parasites the rarer forms, is now realized to be a matter of practical importance to the medical technologist anywhere in the United States.

The absolute incidence of intestinal infection in any given locality cannot be reached without an appreciation of the problem as a whole. Stool examinations, in consequence, must be done as routinely as urinalyses and serologic tests for syphilis. They frequently reveal entirely unexpected findings, just as abnormal con-

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stituents are often found on routine examinations of the urine and the presence of syphilis is often detected by means of a routine Wassermann test.

No one single method of stool examination is altogether satisfactory for the detection of all species or for the identification of the various forms of parasites. It is necessary to be familiar with and to use a diversity of methods, each of which has certain advantages as well as certain disadvantages. Regardless of the method used, a properly prepared specimen will greatly facilitate the examination and will well repay the additional time spent in its preparation.

A single negative stool examination is not sufficient to rule out parasitism. Repeated examinations are frequently necessary for its detection. Some species, such as Enterobius vermicularis, are passed in the feces only infrequently, and others may be present in such small numbers as to be overlooked at any single examination.

The purpose of this paper is to point out some of the characteristic features of the more frequently encountered parasites and to state briefly some of the methods by which detection and identification of the various intestinal parasites may be facilitated. All of them are in daily use in the Laboratory of Parasitology of the Department of Pathology of Charity Hospital of Louisiana at New Orleans, where they have proven of unquestioned value in simplifying the examination of fecal specimens. References to the original sources are appended. The data in the tables represent a composite of material gathered from several sources and from personal experience.

Intestinal parasites fall into two large groups, Protozoa or single-celled animals, which include Amoebae, Ciliata and Flagellata, and Helminths or worms. Four species of Amoebae are important either from the standpoint of pathogenicity or because identification is often difficult and may result in confusion of species (Table 1).

The trophozoites of the Amoebae are irregular, hyaline, single-celled, actively motile parasites. The structure of the single, usually invisible nucleus is characteristic for each species. Motility is accomplished by means of pseudopodia.

Precysts are round or oval, non-motile, hyaline forms. They are smaller than the trophozoites and usually have a single nucleus resembling the nucleus of the trophozoite, but more easily visible. It is impossible to identify the species when the parasite is present only in this form (Table 2).

Cysts are round, oval or ovoid, hyaline, refractile, non-motile, multinucleate forms. The structure of the nucleus is characteristic for each species (Table 3).

The cysts of Giardia and Chilomastix appear as small, clear, hyaline, refractile bodies with a definite cell outline (Table 4). Only the differential characteristics of the cystic stage of these two Protozoa and the vegetative stage of Trichomonas will be considered, as these are the forms most frequently encountered and on which diagnosis must be made (Table 5).

Balantidium coli is the only important pathogenic ciliate of man (Table 6). It is a large, actively motile organism averaging 60-100 μ in length and 40-70 μ in breadth. It is covered with short, delicate, hairlike projections (cilia) which are constantly in motion and which characterize this species. A smaller micronucleus lies in the concavity of a large, kidney-shaped macronucleus, and there are two large contractile vacuoles as well as smaller food vacuoles. At the anterior pointed end is a distinct cleft and at the posterior end is a smaller, tube-like opening. In iodine-stained preparations the internal structure is indistinct and the cilia may appear agglutinated. The cystic stage is rarely seen in man.

Helminths or worms are relatively large and many of them are readily visible to the eye (Table 7). The ova are microscopic but all have very definite characteristics which render identification fairly easy. In this discussion two classes will be considered, Nematodes or round worms and Cestodes or tape worms.

Nematodes are typically elongated, cylindrical, and bilaterally symmetrical. The mouth, which is situated at the anterior end, is frequently provided with hooks, cutting plates, spines or other structures useful for attachment to or penetration of tissues. All nematodes parasitic in man have both males and females, the male usually being smaller than the female (Table 8).

Cestodes are a species of flattened, tape-like forms consisting of

a scolex or head, an attenuated neck, and well developed segments known as proglottids (Table 9). The head, which is the organ of attachment, is characteristic for each species. The neck is the organ of regeneration. The most distal proglottids are hemaphroditic and contain ova. As long as the head remains attached the parasite is capable of regenerating itself. The whole worm is known as strobilia. There are six species which are pathogenic for man and are of sufficiently wide distribution to make their diagnostic differentiation a matter of importance (Table 10).

Technics for Stool Examination for Parasites

Examination of the Gross Specimen: This is best accomplished by transferring all or part of the specimen to a large Petri dish after which it is examined with a hand lens. Suspicious particles are transferred to a slide, a cover glass is applied and the findings are confirmed under the low dry power of the microscope. Ascarids, adult Enterobius vermicularis, and proglottids of the tapeworms are visible macroscopically, and the diagnosis is frequently missed if this part of the examination is omitted.

Direct Smear: Two preparations are made on a fecal slide (1.5 x 3 in.). A small amount of the fecal specimen is emulsified in a few drops of saline on each end of the same slide. To one emulsion is added one to two drops of iodine solution (1 per cent KI saturated with I.) and a cover glass applied. The other preparation is examined untreated. A satisfactory preparation is one through which newsprint appears distinct enough to be read when the cover glass is applied. Trophozoites and precystic forms of the Protozoa are frequently detected by this procedure when they are otherwise missed.

Concentration by Centrifugalization: Sufficient normal saline (tap water may be used) is added to the specimen to make a soupy suspension. The specimen is thoroughly emulsified by stirring with a glass rod or with a tongue blade so that the portion taken for examination will be representative of the whole. About 3-4 cc. of this emulsion are strained through four thicknesses of gauze into a Wassermann tube, which is then filled almost to the top with normal saline solution. The suspension is mixed by inverting the tube several times. The tube is centrifuged at 500-1,000 revolutions for

40-60 seconds. Cysts, ova, larvae, small worms and large fecal fragments will be thrown to the bottom, bacteria and amorphous material remaining suspended. The supernatant fluid is now poured off and the tube is again filled with saline. The contents are thoroughly mixed and are again centrifuged. This procedure is repeated until the supernatant fluid is water clear. After the last centrifugalization the supernatant fluid is poured off, leaving about 0.5 cc. of sediment. A few drops of the sediment are placed on both ends of a fecal slide, to one side of which iodine solution is added. Coverslips are placed on both preparations. Examination is caried out with low and high dry magnifications.

Zinc Sulphate Centrifugal Flotation Technic: The specimen is prepared exactly as in the centrifugalization method and is washed with saline solution until the supernatant fluid is clear. Following the last washing the saline supernatant is poured off and zinc sulphate (specific gravity 1.180) is added until the meniscus is just below the rim of the tube. Sediment and solution are mixed by inverting the tube several times. The tube is again placed in the centrifuge and is run for one minute at 2,000-2,500 revolutions per minute. Zinc sulphate is then added drop by drop until the convexity of the fluid extends very slightly above the rim of the tube. One or two minutes are allowed for all parasites to float to the top, after which a cover slip is applied to the top of the fluid and quickly withdrawn. One or two drops of normal saline solution are added and the cover slip is dropped *preparation side down* on a fecal slide. A second cover slip preparation is made to which one or two drops of iodine solution are added. Both preparations are examined under low and high dry magnification.

The zinc sulphate solution must be prepared so that the specific gravity is exactly 1.180. For this purpose it is necessary to use a hydrometer, the inexpensive type used for checking batteries being satisfactory. Approximately 331.4 grams of U.S.P. granular zinc sulphate per liter of distilled water give a solution having a specific gravity of 1.180 at 26° C in New Orleans.

N.I.H. Swab for Enterobius Vermicularis: The N.I.H. anal swab has been found to yield excellent results in the search for Enterobius vermicularis infections. A glass rod 3-4 mm. in diameter

and 11-12 cm. in length, with rounded smooth ends, is passed through a #00 rubber stopper. A square of cellophane the size of a 7-8 inch cover glass is folded over one end and held in place with a small rubber band, which can be made by cutting a 2 mm. segment from soft gum rubber tubing such as that on blood counting pipettes. The swab is plugged into a test tube to protect the cellophane tip.

Two swabs are usually taken on each patient in the morning before bathing, one from the perianal folds and one within the rectum. The swab is replaced in the test tube until ready for examination.

For the examination, the rubber band is slipped up on the rod with a pair of tweezers. The cellophane square is thus released and is dropped *preparation side up* on a fecal slide. A few drops of N/10 NaOH are placed on the cellophane. A cover glass is applied as soon as the wrinkles have disappeared and the preparation is examined under the low drop objective of the microscope. Care must be exercised that the fingers do not touch the cellophane, since Enterobius vermicularis ova are fully embryonated and infective when removed by the swabs or passed.

Hemotoxylin-Eosin Stain for Amoebae: The staining method is most useful when it is desired to preserve the preparation or for class demonstration. The following technic has been found to give satisfactory results:

1. Fix in hot Schandin's solution to which 5-10 cc. acetic acid has been added. (10 min.)
2. 95% alcohol plus iodine enough to make a port wine color. (5 min.)
3. 70% alcohol. (5 min.)
4. Rinse in tap water. (3 min.)
5. 4% Iron alum. (15 min.)
6. Rinse in tap water. (2 min.)
7. Stain in 0.5% aqueous hemotoxylin. (10 min.)
8. Decolorize in 0.25 per cent Iron alum. (12 min.)
9. Wash in running water. (30 min.)
10. Dehydrate as for paraffin sections, clear and mount in Canada balsam.

At no stage during the staining process should the smear be

allowed to dry.

Culture Method for Protozoa: Occasionally it is desirable to check a diagnosis of cysts or Trophozoites by culture of the stool. Numerous types of culture media have been recommended for the purpose; at times any one will give excellent results, but at other times results are none too encouraging. Boeck and Drbohlav's medium is prepared by washing four eggs, brushing them with alcohol and breaking them into a sterile flask. Fifty cubic centimeters of Locke's solution are added and the mixture is broken up by shaking. About 5 cc. of the mixture are poured into culture tubes and heated in an inspissator at 70° C to coagulate the medium in a slanting position. After coagulation takes place the medium is sterilized in the autoclave for 20 minutes at 15 pounds pressure. A mixture of equal parts of sterile inactivated human blood serum and Locke's solution is prepared. The slants are covered to a depth of 1 mm. with this mixture.

The culture medium is inoculated with a particle of feces about the size of a pea, which is mixed with the supernatant fluid. The cultures are incubated at 37° C and examined after 48 hours. Subcultures may be made at this time.

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TABLE 1

Class	Species	Diagnostic Forms in Stools
Rizopoda Endamoeba	E. histolytica	Trophozoites, precysts, cysts
Rizopoda Endamoeba	E. coli	Trophozoites, precysts, cysts
Rizopoda Endolimax	E. nana	Trophozoites, precysts, cysts
Rizopoda Iodamoeba	I. butschlii	Trophozoites, precysts, cysts

TABLE 2

DIFFERENTIAL CHARACTERISTICS OF THE AMOEBAE TROPHOZOITES (MOTILE VEGETATIVE FORMS) UNSTAINED

Name	Endamoeba histolytica	Endamoeba coli	Endolimax nana	Iodamoeba butschlii
Size	18-45 μ	18-40 μ	6-12 μ	9-18 μ
MOTILITY	Very active, progressive and directional.	Sluggish. Movement usually not progressive.	Sluggish. Movement tends to be progressive.	Sluggish. Movement tends to be progressive.
PSEUDOPODIA	Hyaline, clear, fingerlike projections. Rapidly extruded. Movement sudden. Produces bizarre shapes. Several may be extruded at one time.	Granular, blunt, slowly extruded. Changes shape but slightly, tends to round up.	Hyaline, blunt, slowly extruded.	Hyaline, blunt, slowly extruded.
INCLUSIONS	Red blood corpuscles usually present. Bacteria and particulate matter usually absent.	Red blood corpuscles present only rarely. Bacteria, particulate matter and debris present.	No red blood corpuscles present. Bacteria present.	No red blood corpuscles present. Bacteria present.
VACUOLES	Absent.	Numerous.	Numerous.	Present.
NEUCLEUS UNSTAINED	One present. Usually invisible.	One present. Usually visible.	One present. Usually visible.	One present. Usually visible.
NUCLEAR MEMBRANE (STAINED)	Delicate. Inner layer of very fine chromatin dots.	Thick. Inner layer of large chromatin dots or plaques.	Intermediate. Chromatin granules usually not present.	Thick. Chromatin dots may be present.
KARYOSOME	Single. Centrally placed. Minute dots.	One or more large particles, eccentrically placed.	Single. Large. Eccentrically placed.	Large. Granular. Centrally placed.

TABLE 3

DIFFERENTIAL CHARACTERISTICS OF THE AMOEBAE CYSTS

IODINE STAINED

Species	Endamoeba histolytica	Endamoeba coli	Endolimax nana	Iodamoeba butschlii
Size	6-20 μ	6-30 μ	5-11 μ	5-18 μ
SHAPE	Spherical. Occasionally oval.	Spherical. Occasionally slightly irregular.	Oval, occasionally spherical or ellipsoidal.	Oval, occasionally irregular.
NUMBER OF NUCLEI	1-4. Never more than 4.	1-8. Sometimes more.	1-4. Occasionally more.	1. Occasionally 2.
STAINING CHARACTER OF NUCLEI	Nuclei do not always stain well. May be difficult to identify structure.	Nuclei usually stain well and structure shows up distinctly.	Nuclei stain poorly. Structure difficult to identify. May appear as highly refractile, bright dots or bars.	Nucleus may be indefinite but glycogen mass is well stained and characteristic.

STRUCTURE OF NUCLEI	Membrane delicate. Chromatin granules appear as fine regular dots. Karyosome is a minute, centrally placed dot.	Membrane thick. Chromatin granules appear as irregular masses or plaques. Karyosome is a large, eccentrically placed dot.	Membrane is indistinct. Karyosome is a single or divided, irregular mass; often appears as a small bar.	Membrane very delicate, often indistinct. Karyosome is eccentrically placed.
CYTOPLASM	Finely granular, alveolar, often vacuolated. Stains yellow with iodine.	Coarsely granular, vacuolated. Stains yellowish-brown with iodine.	Finely granular vacuolated. Stains poorly (a very pale yellow) with iodine.	Coarsely granular, vacuolated. If glycogen mass is large there may be only a small rim.
CHROMATOIDAL BODIES IN CYTOPLASM	Bar, oval or rod-like masses with rounded ends.	Rare, filamentous or splinter-like masses with pointed or square ends.	Rare. Small, spherical, often in a vacuole.	Rare, usually absent.
GLYCOGEN MASS	Diffuse. Stains reddish brown with iodine.	Indefinite mass. Stains dark brown.	Usually absent.	Sharply outlined, round, oval or ovoid. Stains dark mahogany brown with iodine.

TABLE 4

FLAGELLATES

Protozoa-Flagellata

Class	Genus	Species	Diagnostic Forms
Mastigophora	Giardia	Giardia lamblia	Cysts, rarely trophozoites.
Mastigophora	Chilomastix	Chilomastix mesnili	Cysts, rarely trophozoites.
Mastigophora	Trichomonas	Trichomonas hominis	Trophozoites, no cystic stage.

TABLE 5

DIFFERENTIAL CHARACTERISTICS OF THE FLAGELLATA

Species	Giardia lamblia cysts	Chilomastix mesnili cysts	Trichomonas hominis Trophozoites
Size	8-14 μ x 6-10 μ	6-9 μ x 4-6 μ	7-15 μ
SHAPE	Oval, ovoid or round.	Pear shaped or irregular, occasionally round.	Actively motile, pear shaped, hyaline organism. Anterior extremity is rounded and has 3-5 flagella. Posterior extremity is pointed. There is an undulating membrane which may be projected as a single posterior flagellum. The nucleus is invisible in motile specimens. As motility ceases the organism tends to round up and the flagella and undulating membrane become invisible.
CYST WALL	Distinct, double outline.	Distinct, single, thin membrane.	
NUMBER OF NUCLEI	2-4. Usually in pairs.	Lying in anterior end or near middle.	
STRUCTURE OF NUCLEI	Small, round, clear, distinct. Karyosome eccentrically placed.	Round or oval with thin, distinct membrane. Karyosome is eccentrically placed.	
RETRACTED FLAGELLA	Distinctly visible. Short fibrils also present.	Visible but less distinct. Two fibrils also present.	
IODINE STAIN	Stains distinctly, yellowish brown. Internal structure becomes more distinct. Cytoplasm shrinks away from cyst wall.	Stains very pale, yellowish. Internal structure becomes slightly more distinct.	

TABLE 6

CILIATES

Protozoa-Ciliata

Class	Genus	Species	Diagnostic Form
Ciliata	Balantidium	Balantidium Coli	Trophozoites. Rarely cysts.

TABLE 7

HELMINTHS

Nematodes—Round Worms

Common Name	Class	Super Family	Species	Forms found in Stool
Whip worm	Nematoda	Trichinelloidea	Trichocephalus trichiurus	Ova
Round worm	Nematoda	Ascaroidea	Ascaris lumbricoides	Ova, larvae, adult worms
Pin worm	Nematoda	Oxyuroidea	Enterobius vermicularis	Ova, larvae, adult worms
Hook worm	Nematoda	Strongyloidea	Necator Americanus	Ova, rarely rhabditiform larvae
	Nematoda	Rhabditoidea	Strongyloides stercoralis	Rhabditiform larvae, rarely ova

TABLE 8

CHARACTERISTICS OF THE MORE COMMON NEMATODES

Name	Stage	Size	Color	Shape	Internal Appearance	External Appearance
TRICHO-CEPHALUS TRICHIURUS	Ova	22x50 μ	Brown	Symmetrical. Barrel or lemon shaped.	Contains immature unsegmented embryo.	Has double wall. Outer wall is darker brown. Mucoid plug at either pole.
ASCARIS LUMBRI-COIDES	Ova (fertilized)	45 - 75 μ in length 30-50 μ in diameter	Brown. Occasionally grayish brown.	Broadly ovoid or round.	Embryo unsegmented contains mass of lecithin crystals. Occasionally appears as a granular mass.	Thick, transparent shell with coarsely mamillated albuminous outer layer.
	Ova (unfertilized)	80 - 95 μ x 35 - 45 μ	Brown or grayish.	Ovoid, round, irregular or bizarre.	Contains mass refractile granules.	Outer mamillated coat may be absent or mamilations may be irregular in size.
	Adult worm	This is the largest of the common intestinal parasites. The male is 15-31 cm. in length, female 20-50 cm. The posterior extremity of male is curved. The color is brown to grayish. The worm resembles the common earth worm.				

(Continued on next page)

NECATOR AMERICANUS	Ova	40x70 μ	Colorless or Grayish.	Oval	Segmented or mature embryo present. In fresh specimen embryo may be motile. In degenerating ova may appear as a granular mass.	Single thin shell wall. Clear space surrounding embryo.
STRONGYLOIDES STERCORALIS	Rhabditiform larvae	20x400 μ	Colorless	Long, narrow with distinct tail. Actively motile in fresh specimen.	Has very short buccal cavity and inconspicuous genital pore, difficult to see in unstained specimen. Easily distinguished in iodine stained preparation.	
ENTEROBIUS VERMICULARIS	Rhabditiform larvae	20x400 μ	Colorless	Long, narrow, with distinct tail.	Has long buccal cavity and prominent genital pore. Actively motile in freshly passed specimen. Degenerates fairly rapidly. Iodine stain necessary to accentuate internal structure.	
	Ova	25x50 μ	Colorless	Elongated, ovoid. Flattened on ventrical surface.	Contains well developed coiled embryo.	Shell thick, has double contour. Embryo fills shell.
	Larvae	Small, white, actively motile in fresh specimen or before drying occurs. Has cuticular expansions at anterior end. Posterior end is attenuated and pointed.				
	Adult female	10-12 mm in length	White, actively motile in fresh specimens. Uteri of gravid females filled with embryonated ova. Cuticular expansions at anterior end. Posterior end pointed. Pressure of cover slip on drying causes uteri to contract and expel ova.			
	Adult male	2-5 mm in length	White, movement very slight and sluggish. Has cuticular expansions at anterior end. Posterior end sharply curved ventrally.			

TABLE 9

CESTODES — TAPEWORMS

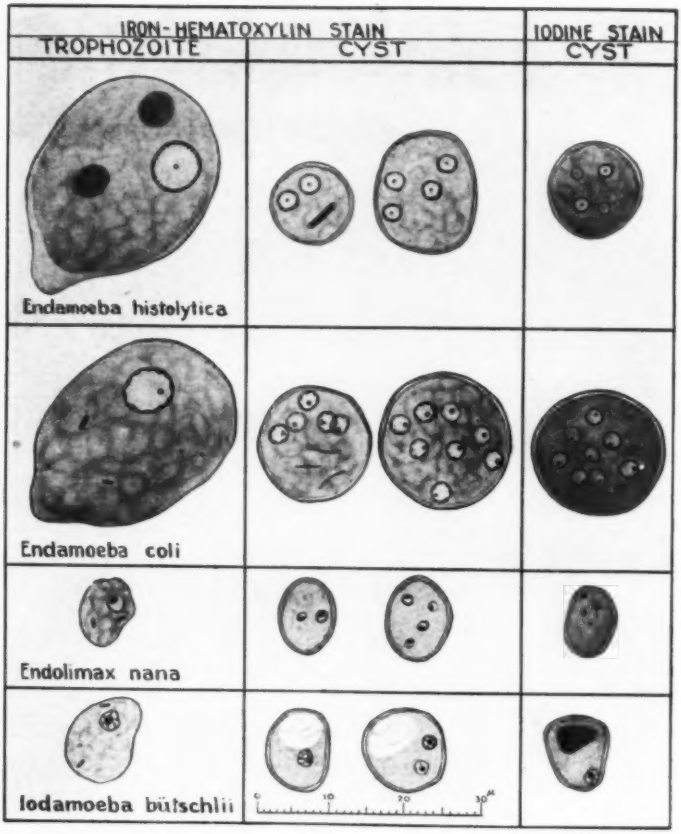
Common Name	Class	Super Family	Species	Forms found in Stool
Beef tapeworm	Cestoida	Taenioidea	Taenia saginata	Ova, proglottids
Pork tapeworm	Cestoida	Taenioidea	Taenia solium	Ova, proglottids
Dwarf tapeworm	Cestoida	Taenioidea	Hymenolepsis nana	Ova, proglottids
Rat tapeworm	Cestoida	Taenioidea	Hymenolepsis diminuta	Ova, proglottids
Dog tapeworm	Cestoida	Taenioidea	Dipylidium caninum	Ova, proglottids
Broad or fish tapeworm	Cestoida	Bothriocephaloidea	Diphyllobothrium latum	Ova, proglottids

TABLE 10

DIFFERENTIAL CHARACTERISTICS OF THE CESTODES

Name	Length of adult worm	Scolex	Proglottids	Ova
TAENIA SAGINATA	200-400 cm	Unarmed. Has 4 suctorial discs. No rostellum.	1000-2000. Uterus in gravid proglottids has 15-30 main lateral branches.	Average 35 μ in diameter. Oval to round. Single. Thick wall with radial striations. Brown. May be enveloped in thin hyaline membrane having a pair of delicate filamentous processes. Ova contain hexacanth (6 hooklets) embryo.
TAENIA SOLIUM	180-250 cm.	Armed. Has 4 deeply cupped suctorial discs and a rostellum armed with a double row of hooklets 22-32 in number.	100 or less. Uterus in gravid proglottids has 7-13 main lateral branches. Ova are set free from uterus through ventral longitudinal slit.	Indistinguishable from T. saginata.
HYMENOLEPSIS NANA	2-4 cm.	Armed. 4 suctorial discs. Short rostellum armed with a single row of 20-30 spines.	200 or less. Eggs are set free by disintegration of distal gravid proglottids.	Average 40 μ in diameter. Round, grayish to colorless. There is an inner and outer hyaline membrane. There are two polar thickenings from which arise 4-8 polar filaments. Ova contain a hexacanth (6 hooklets) embryo. Hooklets easily seen.

HYMENOLEPSIS DIMINUTA	20-60 cm.	Unarmed. 4 deeply cupped suctorial discs. Pyriform rostellum without hooklets.	1000 or less. Ova discharged when gravid proglottids disintegrate.	Average 70 μ in diameter. Ova. Have transparent yellowish outer shell and an inner membrane around hexacanth embryo. There are two polar thickenings but no filaments. Hooklets arranged in fan shape.
DIPYLIDIUM CANINUM	10-50 cm.	Armed. 4 deeply cupped suctorial discs, club shaped rostellum armed with 3-7 rows of spines.	200 or less. Melon seed shaped, contain ova in embryonic membrane which are set free when proglottids disintegrate.	Average 35 μ in diameter. Round, thin shelled, hyaline. Pass in blocks of 8-15 enclosed in embryonic membrane.
DIPHYLLOBOTHRIUM LATUM	30-100 cm.	Unarmed. Has lateral suctorial grooves.	3000 or more. Ova are deposited regularly through uterine pore.	Average 45x70 μ. Large, ovoid, yellowish brown, granular. Wall is double contoured. Characteristic feature is operculum. Ova contains immature embryo.


IRON-HEMATOXYLIN STAIN
IODINE STAIN
TROPHOZOITE
CYST
CYST
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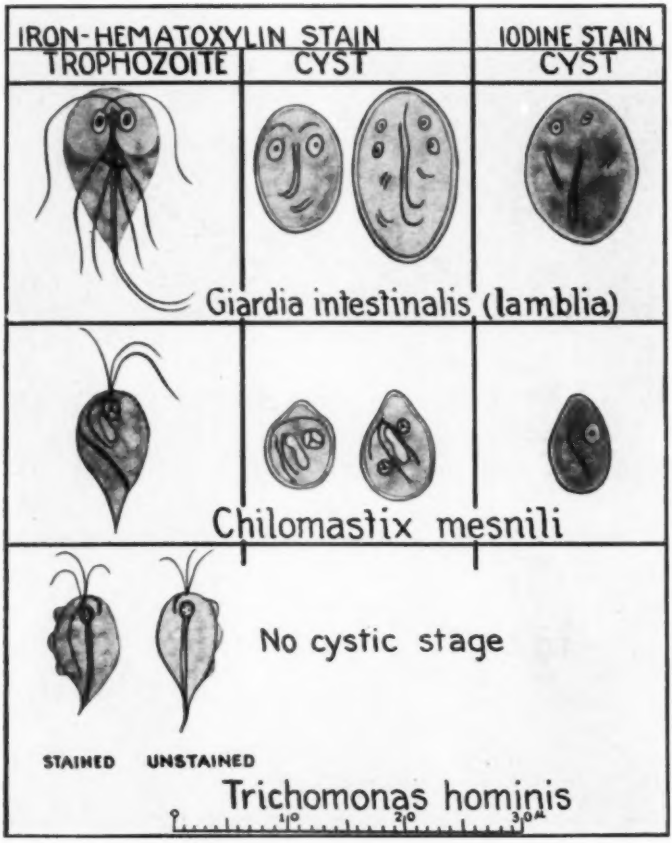
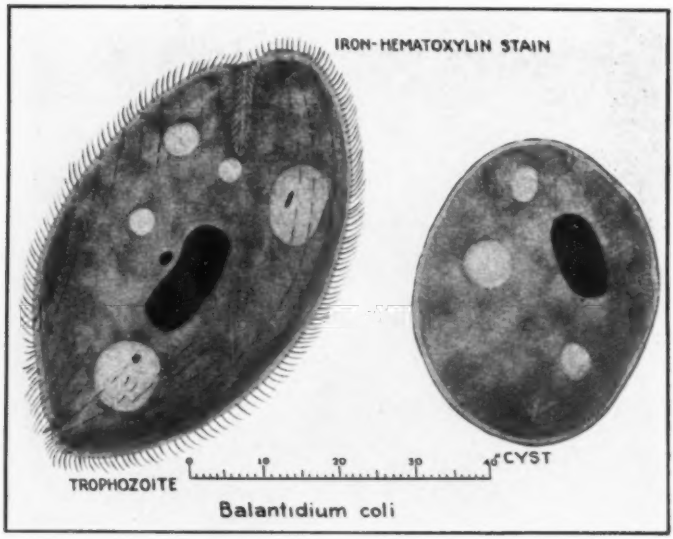
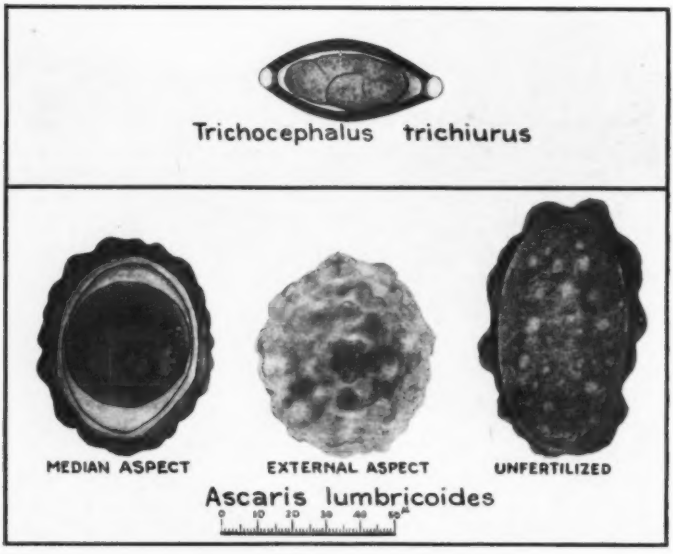
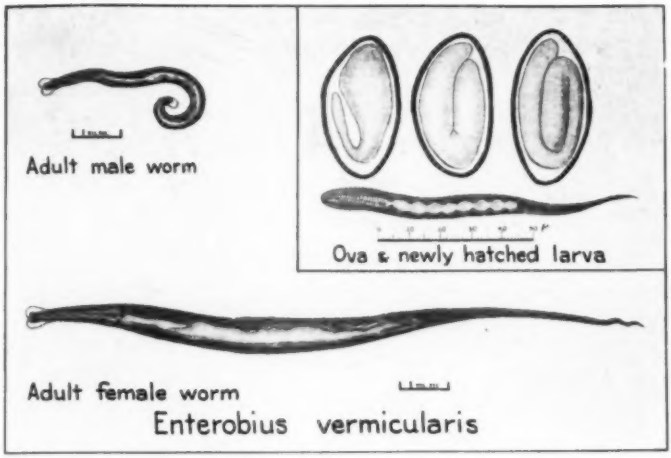
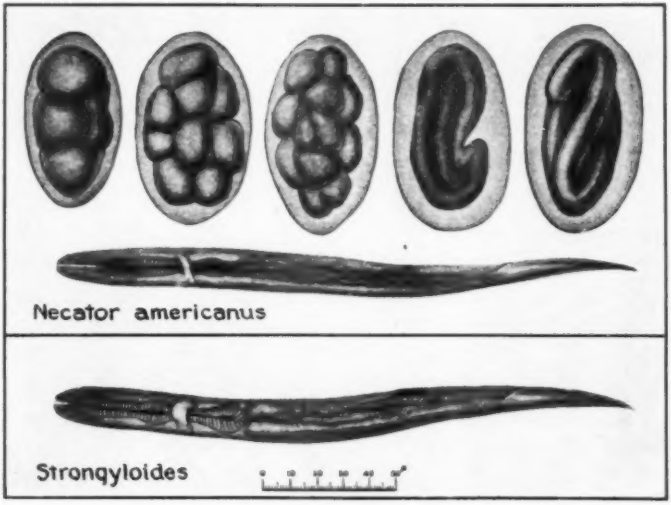
Endamoeba coli
Endolimax nana
Iodamoeba bütschlii
0
10
20
30 μ

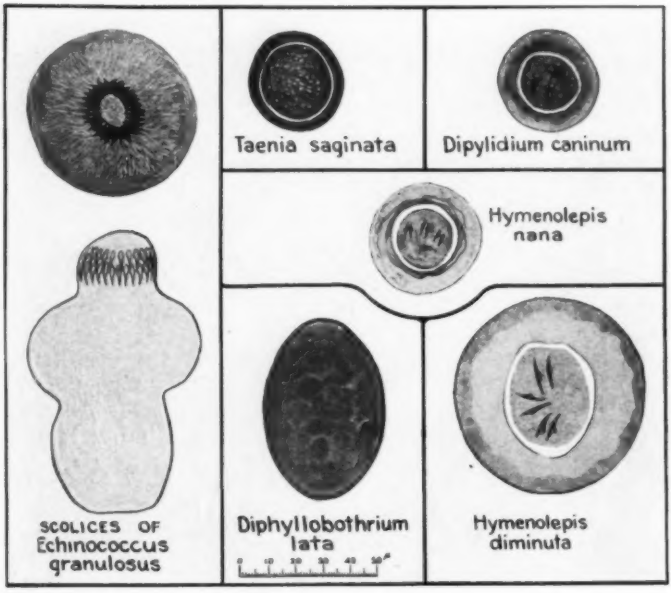

IRON-HEMATOXYLIN STAIN
TROPHOZOITE
CYST
IODINE STAIN
CYST
Giardia intestinalis (lamblia)
Chilomastix mesnili
No cystic stage
STAINED
UNSTAINED
Trichomonas hominis
1μ
2μ
3μ


IRON-HEMATOXYLIN STAIN
CYST
TROPHOZOITE
Balantidium coli


Trichocephalus trichiurus
MEDIAN ASPECT
EXTERNAL ASPECT
UNFERTILIZED
Ascaris lumbricoides


Adult male worm
Ova & newly hatched larva
Adult female worm
Enterobius vermicularis


Necator americanus
Strongyloides



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A TECHNIQUE FOR ROUTINE BLOOD CELL COUNTS USING OXALATED BLOOD

By EVELYN N. JARDINE, M.T.

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Time does not permit me to review with too much detail the devious paths our instructors have taken and are still taking to lead us into the most efficient technique possible in the method of blood counting with anti-coagulated venous blood.

To touch a few fo the high spots: it was in 1933-34 that several papers were written and published advocating the use of venous blood because of greater accuracy. Certain it is that everyone here today has seen the anemic patient or the poorly punctured ear or finger being milked and wrung like the good old dishcloth for every bit of lypmh within an inch of the puncture! It is reasonable to expect that blood taken from the vein reaches our hands freed from the human element of poor technique in obtaining the specimen.

All the earlier literature on the subject of anti-coagulants for blood was based on heparin as the ideal, and oxalates were run for comparison. The test used was the sedimentation rate.

There was no definite agreement among these writers regarding the amount of oxalate for each cc blood.

Heparin itself is out of the race for favor because of its expense. One of the writers in 1933 stated that heparin contains a calcium contamination, does not dissolve to dryness, and the blood has a tendency to form small clots about the heparin. Whether this is true matters little, because heparin is not practical for routine laboratory work. I merely mention heparin because it was the anti-coagulant from the use of which such men as Drs. Heller, Paul, Hasking, and Wintrobe reached their conclusions concerning the ideal anti-coagulant for blood.

In deciding upon which formula to use for an anti-coagulant, a technician turns naturally to the textbooks recommended by our Board of Directory. Upon doing so, one finds quite a wide choice, as there are practically no two books agreeing as to the strength of the solution of potassium or sodium oxalate. One might therefore conclude that it cannot make any difference which anti-coagulant is used, were it not for the table in Kolmer and Boerner's second edition, which gives .1 cc of 2% potassium oxalate per cc of blood evaporated to a dryness, designating the hours after taking within which the blood work must be done. I have as yet not seen the research work from which this table was compiled. But certain it is that when technicians see such a table they immediately sense that there is more to this method than merely having the blood unclotted, and it is just as apparent that information of a definite kind is required before interpretations can be made.

From the long list of anti-coagulants a choice was made at the laboratory of the Mary Hitchcock Memorial Hospital, Hanover, N. H., of a formula mentioned first in 1933 by Drs. V. G. Heller and Henry Paul of Oklahoma and since by Dr. Wintrobe.

In the *American Journal of Medical Sciences*, in 1934, Dr. Wintrobe made this statement:

"Following the suggestion of Heller and Paul we have commenced to employ as an anti-coagulant a mixture of ammonium and potassium oxalate (6 mg of the former and 4 mg of the latter per 5 cc blood). In these proportions no difference in the volume of packed red cells as compared with the volume in heparinized blood has been noted and consequently no correction for shrinkage is necessary."

From 1934 this same strength solution has been frequently mentioned in the literature with apparent growing favor.

In *A Textbook of Clinical Pathology*, by Dr. Roy Kracke, the statement is made that this proportion of solid potassium and ammonium oxalate will prevent coagulation without shrinkage or alteration of sedimentation rate.

A brief and not too technical explanation offered by chemists of just what needs to be considered when we prevent the clotting of blood with oxalates is as follows:

When fixed weights of potassium and ammonium oxalate are brought into a given volume of aqueous solution an ionic equilibrium is established which is independent of the method of mixing the salts. The evaporation of aliquote parts of standard solutions is universally adopted because it is the most convenient and accurate procedure.

Used as an anti-coagulant in the preparation of blood samples for routine laboratory work, the oxalate ion alone is active in removing ionic calcium as insoluble calcium oxalate. Therefore, either salt or any mixture of them will suffice provided enough is used to accomplish this purpose.

However, it seems clear that variations in the ratio of the salts might affect the formed elements in accordance with laws of osmosis, by involvement of two variables.

First, the pH of the solution might be lowered by the hydrolysis of the ammonium salt, since ammonia is a weak base and oxalic acid a moderately strong acid. No considerable lowering would be expected in a well-buffered solution like blood even when strongly diluted. Its extent might be measured by methods susceptible of very great accuracy.

Secondly, by virtue of the relative permeability of the cell wall of the element to the ammonium and potassium ions osmotic conditions would change with varying concentrations of these ions. If the lighter and speedier ammonium ions found their way into the elements more readily water would dialyze in with them. This would bring about a definite change in the size and make-up of the element.

The sum total of these changes will not be shown without much research, but I have attempted to accumulate some data on routine work to assist us in interpretations.

A series of blood sugars run as comparison between this recom-

mended formula and the usual 1 to 2% potassium oxalate in the chemistry collection tube showed no appreciable difference.

These figures do not vary more than is accountable from the human element involved. Therefore it is safe to say that one may take from the blood collection tube, after the count is assembled, enough blood for a sugar determination. This also was done with the sulfanilamide determination and found to be accurate, which is very saving on patient and time because of daily counts usually ordered with this drug. I insert this because from experience after receiving the routine admittance urine, complete blood count and Wassermann and Kahn, and upon the urine showing sugar, an order for a stat blood sugar has usually followed. The chemistry department has used the blood remaining from the count, thus saving the patient a second vena puncture and also the technician's time.

On the contrary such an exchange is not permissible in determinations in any way affected by ammonia. I refer in particular to Nesslerized determinations.

A table indicates what happens in the case of a non protein nitrogen determination of blood anti-coagulated with the ammonium mixture:

TABLE I

Test	Dilution 1.2 .8 $(NH_4)_2K_2C_2O_4$	Dil. 1.1% $K_2C_2O_4$	Dil. 1.6% $K_2C_2O_4$	Dil. 2% $K_2C_2O_4$
NPN in Mgs	75.0			52.0
	63.0			33.0
	68.0	36.0	31.3	33.4

Table I. Ammonium and Potassium Oxalate Mixture when Nesslerized.

A series of fragility tests furnished the interesting data that one need not add the drops of blood to the tubes directly from the needle at the bedside. The results were no different when taken from the blood collection tube. Even five hours after collection of blood the results were the same.

TABLE II

Time	Dilution 1.2 .8 $(NH_4)_2K_2C_2O_4$	Dil. 1.1% $K_2C_2O_4$	Dil. 1.6% $K_2C_2O_4$	Dil. 2% $K_2C_2O_4$
	E. S. R.	E. S. R.	E. S. R.	E. S. R.
Taking	12	8	8	9
24 hrs.	0	0	0	0

Table II. Comparison of recommended dilutions to sedimentation rate.

This illustrates the difference in sedimentation rate of different potassium oxalate concentrations.

TABLE III

	Time	Taking	5 hours	24 hours	White Blood Count
SPECIMEN	1.	3.2	3.2	1.0	8,050
	2.	1.0	1.0	0.0	12,200
	3.	100.0	20.0	3.0	26,000
	4.	22.0	19.0	2.0	12,400

Table III. Stability of sedimentation rate in normal count. Source of error in elevated count.

Notice that specimen I is that of a healthy normal person in which the sedimentation rate may be said to hold reasonably well while the specimen with an elevated count shows a considerable change to be apparent. Why it does not always happen I do not know. I am very much intrigued with the thought that in these cases one might find an abnormal condition affecting the pH of the blood. All I can say is that there are such specimens, therefore one must treat every specimen with equal caution. Possible source of error can be eliminated by setting up the sedimentation rate within the hour.

TABLE IV

Time	Dilution 1.2 .8 $(NH_4)_2K_2C_2O_4$ White Blood Count	Dil. 1.1% $K_2C_2O_4$ White Blood Count	Dil. 1.6% $K_2C_2O_4$ White Blood Count	Dil. 2% $K_2C_2O_4$ White Blood Count
Taking	10,300	9,200	9,200	9,500
3 hrs.	9,200	8,100	7,400	8,200
5 hrs.	9,400	8,100	7,500	6,400
24 hrs.	8,900	7,200	7,400	7,900

Table IV. Behavior of white blood count over twenty-four hour period with comparison of recommended dilutions.

In order to give a true interpretation blood was taken from a patient and exactly 5 cc was placed in each of the tubes with these designated dilutions of anti-coagulant. The counts were assembled and counted with the same technique at approximately the same time with this result. I pick this case because it is typical of 49 specimens from the fifty that were carried through.

This recommended dilution gave a higher count in all but one instance. The greatest difference in 24 hours averaged 1,000 cells. The other dilution showed from 900 to 2300, the average being about 1500 cells in 24 hours. In cases of normal bloods the change was very slight, but in most cases where a toxic condition existed the count decreased more rapidly. No very great difference occurred under five hours, therefore one might conclude the count to be fairly accurate within that time.

The question of a differential is an even more delicate matter.

A series of over fifty differentials showed in some cases a change after the first hour and again very little change in 3 hours. In every instance, vacuolated white blood cells and some degenerated cells appeared after 3 hours. Again, in cases of high white counts the degeneration was faster. Also the mononuclear leukocytes and

lymphocytes assumed bizarre nuclei that were not in evidence with the first hour. In 24 hours it was not uncommon to see the polymorphonuclear leukocyte count drop to half its original number, and in some cases the large number of degenerated cells would make it practically impossible to count. Again, in cases of high white counts the degeneration was faster. This tendency of polymorphonuclear leukocytes to decrease and the lymphocytes to assume bizarre nuclei might be to the uninitiated misleading.

The conclusion, therefore, in regard to differentials, is that they should be transferred to a smear as soon as conveniently possible, due to the fact that vacuolated cells, bizarre nuclei, and crystals will appear and often the polymorphonuclear leukocytes will degenerate rapidly.

The actual solution is made by dissolving 0.8 gms potassium oxalate and 1.2 gms ammonium oxalate in 100 cc of distilled water. Of this solution 0.5 cc is placed in each vial intended to receive the blood specimen. The vial is now placed in a drying oven and the solution slowly evaporated to a dryness. The vial will now contain 4 mgms of solid potassium oxalate and 6 mgms of solid ammonium oxalate and is ready to receive the blood specimen.

The second essential point of technique is that exactly 5 cc. of blood is to be added to this vial and the elements mixed gently for one minute. You will recall that Drs. Wintrobe and Kracke both stated "In these proportions" there will be no shrinkage of packed red cell volume or alteration of sedimentation rate.

It is advisable to use a vial about 1½ inches in height and of sufficient diameter to contain about 7 cc of blood. This type of vial is not easily tipped over, and yet a dozen or more can be carried easily on the serology or venous puncture tray. This enables a technician to be back in the laboratory with eight or ten complete counts with serology in a very short time. While these counts are being assembled, by one technician, another one may be out obtaining more specimens.

The next point of technique that is absolutely essential is that of mixing the elements in the vial before assembling blood counts.

It is necessary that the contents of the vial be mixed gently for a minimum of one full minute. Two minutes is not too long, but at least one is absolutely essential. Mix with a steady even motion. Do not shake.

The usual technique of assembling the red, white, hemoglobin and differential is observed with these advantages: one is seated at a convenient height bench, light is such that there is no danger of not seeing the measurements correctly on the pipette, all elements that distract and interrupt at the bedside are removed, mistakes can be rectified until a correct count is obtained.

After the count has been assembled correctly, the pipette must be shaken three minutes by clock before the counting chambers are filled. The usual checking margins are observed with the great advantage that failure to check can further be verified by going back to the original blood specimen. The following points are obviously in favor of the use of venous blood for counts:

If you have a school for technicians there is no more efficient way of demonstrating technique than in the laboratory with oxalated specimens. When practicing the student can be checked daily in her efforts by the technician's report given on the same blood sample.

Two years' experience with this method finds it popular with the professional staff and with the laboratory group. It gives the staff man a chance to add an order overlooked with the routine order, and it gives the laboratory a chance to correct an accident without disturbing the patient.

Summary

That 0.5 cc of a solution containing 0.8 gms of potassium oxalate and 1.2 gms ammonium oxalate evaporated to a dryness will contain the necessary amount of solid ammonium and solid potassium oxalate to prevent 5 cc of blood from coagulating without shrinkage or damage to the cell elements.

5 cc of blood in above anti-coagulant will give an accurate blood count for a period of 5 hours with the exception of the differential and sedimentation rate, which should be within the first hour.

Blood sugars and sulfanilamide determinations can be done from this sample, but not determinations requiring a nesslerized solution.

Accurate results can only be obtained by carefully observing the proper technique as outlined.

Conclusion

Use of oxalated venous blood is the most accurate and efficient method of obtaining correct blood counts, if proper time allowances are observed.

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THE GOOD AND BAD OF HISTOLOGICAL TECHNICS*

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There is scarcely any technical work done in a hospital laboratory which is more important than the preparation of good slides for tissue diagnosis. Decisions of the greatest importance to the patient are made on the basis of what these slides show. From the pathologist's point of view, of course, it is necessary for a really intelligent opinion to be given that there be a full exchange of information between the clinicians who have charge of the patient and the pathologist. This intelligent cooperation should be transmitted between the pathologist and the technician who makes the slide.

Generally speaking there are two main types of work necessary in preparing these tissue slides. The one large type comes from autopsies and the other from living patients either in the form of biopsies or in the form of tissues removed at operation.

Pathology, be it understood, is not in the realm of an exact science; a very large part of it is art. Like every other art it requires years of application before that elusive thing called experience leads to a sort of mental understanding and a feeling of being at home with tissues. What the pathologist really does is have stored in his memory a vast series of pictures of what the various parts of the body look like when they are normal and of how they are changed when disease makes its appearance.

A word or two about the word "normal" is not out of place. Since human beings are living dynamic entities, it is quite apparent that there is no one picture which can be considered normal in practically any part of the body at any time, for that "dynamic equilibrium called life" is in fact dynamic, and thus the pathologist must have a series of pictures in his mind as constituting the normal. As an example, take the thyroid gland. What constitutes a normal

* *Read at the meetings of the Technicians Institute in Philadelphia, March 11th and 12th, 1940.*

thyroid gland can be told only in terms of a number of different appearances. The pathologist's knowledge of physiology and even chemistry leads him to know that those of us who live along the seacoast have thyroid glands which are different in appearance, in weight and mass from the thyroid glands of those who live away from the seacoast, especially those in so-called goiter districts. He must, therefore, know that the appearance of the thyroid gland of an individual in a so-called goiter district is not normal when it would be normal if it came from an individual living along the seacoast. Furthermore, such perfectly normal functions as puberty, menstruation, pregnancy, all influence the appearance of the thyroid gland. I could multiply this example by many but as a text I believe it illustrates to you the principle that there is no such thing as a normal but that a number of conditions depending on all sorts of circumstances are normal in any particular given individual, or part of an individual.

So the pictures which the pathologist calls up from his memory as he looks at a slide are very numerous indeed, and his judgment is tempered by very many factors of all sorts and descriptions.

It has been said by a very famous pathologist, not long since departed, that what the pathologist needs is a low power lens and a high power brain, rather than a high power lens and a low power brain, which brings forcibly to your attention the fact that architecture is of foremost importance in pathology and that in many cases cellular detail is of secondary value. This is not to deprecate the importance of cellular detail nor does it mean that cellular detail should not be in the technician's mind when he or she prepares the slides. This is not only because it is impossible to obtain good architectural detail without cellular detail but that sometimes indeed the recognition of cells is part of the pathologist's job.

To lead to the question of autopsies. One of the great German pathologists, Chiari, had emblazoned in Latin over the door of his autopsy room the following words, "Here is where the dead teach the living." Pathology starts with the appearance of organs when diseased. From their appearance the sequence of events is deduced which lead to those appearances. This means that it is possible very often to construct a picture of the clinical course of particular diseases from the appearance of the organs after death. I leave it to you to conclude how this type of work has laid the foundations

for all modern medicine. Of what use are theories of cause in relation to a certain condition if that certain condition really doesn't exist, and Pathology tells us what really does happen and puts for us into the correct pigeon-holes the combinations of appearances from which physiology, chemistry and other of the sciences can start in their quest for control of disease. Need I call your attention to the importance of first class technical work in producing slides for this most creative purpose with its two angles, the obtaining of more knowledge than we have at present and the crystallizing of the knowledge that we have into the minds of students who are to be our future doctors and who will carry on for us?

Such matters as accuracy of vital statistics, the gathering of data for life insurance companies with their inevitable premium rates are but other of the numerous important points elucidated by pathology and good slides.

Now from the point of view of the living patient. I take particular zest in using cancer as a text for the only treatment of cancer as you know is radical. No pussy-footing is allowed when the diagnosis is made; the only cure is to remove the malignancy in toto or destroy it utterly and completely. On the basis of a histological diagnosis rests the decision as to whether to remove arms or legs or breasts or the contents of the pelvis. Surely these are indeed weighty decisions.

All of our knowledge and all of our propaganda to you as parts of the medical profession and the laiety alike in the cancer field are considered on the slogan "Diagnose cancer early." The modern doctor when consulted by a patient with a little lump or a little sore somewhere or other says to that patient, "I am so glad that you have come to me early, for I cannot tell by looking at your lump or sore whether it is cancer or not."

Many patients are astounded at this seeming admission of ignorance on the part of the doctor.

"What shall be done?" asks the patient.

"A piece will be removed," says the doctor, "and this will be submitted to a pathologist for an opinion. If I could tell that your trouble is cancer from merely looking at it, then the chances are it would be too late to really cure it, so I am very glad that you have come before I can tell what your trouble is."

The implications of this to the technician are obvious. The

responsibility of making the diagnosis is a very grave one, and the technician shares in it. Indeed the pathologist too is glad when the diagnosis is not obvious, but in order to make this momentous decision he must have the very best of preparations to decide.

And now when live masses of tissue have been removed and the diagnosis of cancer has been made clinically, it must nevertheless still be confirmed through the microscope. And so again sections are prepared. On the diagnosis and sometimes on the details of the diagnosis are based decisions as to further operations, irradiation treatments and other such affairs which are of the utmost importance to the patient. Not only does it interfere and ofttimes take him or her away from their occupation, bread-winning or not, but the very life of the patient depends upon it.

Now as to technic. What does the pathologist want when he asks for an ideal section? He wants one which has been fixed early, thoroughly and properly. In the matter of mitosis, or cell multiplication, for which he hunts many times, remember it takes from twenty minutes to forty minutes for a mitosis to be completed, and that cells in various stages of this process when removed from the body will continue until the mitosis is ended, whereupon of course no others will appear. The function of fixatives is to stop all chemical processes instantly and immediately in the tissues when they are submerged in them. To take big, thick pieces and place them in fixative is to have the outer surfaces fixed promptly and the inner parts slowly, perhaps not for hours. So thin slices must be dropped into the fixative, whatever one is chosen. It makes no difference how large a surface is exposed; it is the thickness of the slice which determines the promptness of complete fixation throughout the piece of tissue. Remember there is a delicate osmotic balance in tissues and if fixatives too acid or too alkaline are used this disturbs relationships, makes artefacts appear within the cells, causes a spurious edema and cloudy swelling so-called, especially if fixation is not very prompt.

The essential action of a good fixative is that of coagulating and rendering insoluble certain constituents of the tissues. It is necessary to preserve all the elements which it is desired to fix in the tissues, but preservation is not alone sufficient. A good fixative must also give good optical differentiation and have sufficient power of penetration to insure fixation throughout depth of the sections

which are trimmed of a suitable thickness for manipulation of the microtome. Since no single chemical substance fulfills all these requirements, most of the best fixing agents are mixtures. Important among these are Zenker's, combining mercuric chloride and a bichromate with the addition of a small proportion of formol or acetic acid; Bouin's, combining formol and picric acid with addition of a small proportion of acetic; and as a simpler fixative, diluted formalin. The first two possess certain superiority in differentiation and in keeping open the possibility for the use of a variety of special staining methods. They possess however a slight but definite disadvantage in requiring a definite time limit for the period of fixation, which is not so strictly limited in the case of formalin. The latter is therefore sometimes to be recommended for greater convenience. After tissues have been fixed it is of considerable importance that the tissues be trimmed before they are dehydrated. There are several obvious advantages to trimming the pieces of fixed tissue. Needless to say trimming should be done by the pathologist. It enables him to be sure the tissue is properly fixed before additional procedures in making the slide are begun. It also enables him to get a better understanding of the gross appearance and topography of the tissue which is subsequently to be studied with a microscope. It reduces the amount of time used by the technician in cutting down to the desired level when the tissue is being cut on the microtome. By making nicks on the surface of the piece of tissue opposite from the surface from which the subsequent section will be taken the pathologist can eliminate uncertainty on the part of the technician regarding the surface from which the technician will cut the section. Whenever possible the shape of the surface from which the section will be cut should be a simple geometrical surface such as a rectangle, square, triangle. It should be small enough so that it will easily be covered by the smallest type of cover slip. Larger pieces can usually be judiciously cut into smaller pieces without destroying topography. I am sure most technicians will agree that it is easier to cut two small pieces than one large piece of tissue.

With most dehydrating agents it is necessary that the fixed tissue be subjected to washing with water. Zenker fixed tissue requires from eighteen to twenty-four hours' washing, and thus adds one day to the time of preparation of a slide. Formalin fixed tissues require only a short period of washing usually from fifteen to thirty

minutes depending upon the size of the pieces of tissue.

Dehydration may be accomplished by the use of a series of alcohol solutions increasing the strength as one goes along and thus prepare the tissues for successful infiltration with paraffin. In recent years dioxan has come into use in many laboratories, and it is truly one of the most effective and most economical methods of dehydration that we have today. I might say that in my own experience it has proved to be a superior dehydrating agent and the impregnated tissues seem less brittle and as the paraffin ribbon is being cut the tissue hangs together better. Dioxan should not be handled carelessly however, and as its fumes are rather toxic, dioxan containers should be tightly covered. Infiltration of tissues with paraffin can be successfully accomplished only after thorough dehydration. There is no difficulty whatsoever with paraffin infiltration in tissues dehydrated with dioxan. After the tissues are thoroughly infiltrated with paraffin, they must be embedded and it is now that the rewards of careful trimming and careful marking become evident as there is no time lost in deciding from which surface the pathologist wishes to have the section cut. There are various ways of successfully embedding. Some prefer small paper cups; others prefer pieces of metal shaped like a carpenter's square so that a solid will be formed shaped to suit one's desires. Cooling must take place rapidly after the melted paraffin is poured to form a block and only experience will aid in determining at what rate of speed one can embed. After the paraffin block is cool it is ready to be further trimmed and then attached either to a metal object holder or a corrugated paper object holder. After it is attached to the object holder it is ready to be cut with the microtome.

And now the knife. Paragraph upon paragraph could be written upon how to treat knives with proper respect. Remember that the steel in knives can be tempered to various degrees of hardness and an ideal temper for all types of tissue has not as yet been achieved. It is well to have several knives of various degrees of hardness for different types of tissue. A knife is a knife and not a saw, not that microtome knives are ever real saws for I doubt if they are good for sawing, but they do have nicks in them which are transferred to the tissues to the utter confusion of detail and smooth sections. Take out the nicks and then use a strop. Have the knife so that it cuts a hair when lightly touched to its edge. Do not be afraid of

assisting your eye with a lens to see if the knife edge is real smooth and without nicks, and then proceed with your cutting. Modern microtomes are wonderful precision instruments. They need oil occasionally and they need occasional adjustment. These are extremely easy things to do and important for the final section.

In staining with two dyes ordinarily one is basic and one is acid and these in turn react with the acid and basic groups respectively in the tissues. Primarily in the tissues the acidic groups are carboxyl and nucleic acids; the alkaline groups are amino, etc. The nucleus takes a basic dye, therefore, it is acid, the cytoplasm takes the acid dye, therefore it is basic in reaction. These reactions should be sharply separated, and when they are, we say that the tissue is well differentiated.

There are of course the special stains which are designed to throw into relief certain special kinds of cells or tissues. Thus there are the bacterial stains for discovering tubercle bacilli, streptococci and a host of others. Then there are such stains as differentiate smooth muscle from connective tissue, or as another example, the silver preparations which pick out nerve fibres, fibrils and even structures in nerve cells. In general the use of these special stains demands a rather special experience. Perhaps it is because they are not used so often that ordinarily they are not as good as they might be. In any case it is essential that control slides be stained at the same time that those under examination are prepared. Thus can be told at a glance whether or not the stains are really working. Remember the old saw about the biologist who said when his wife gave birth to twins "Thank goodness I can use one for control."

Finally it must not be forgotten that these stains are designed to take advantage of special chemical reactions and thus ofttimes require that the tissues be fixed in special solutions.

There is an old adage in surgery which says "Diagnose well, cut well, sew well and the patient will get well." To paraphrase all that I have said in our vernacular, or to pour a fixative over it so that it will congeal in your minds, let me conclude then by stating that meticulous attention to detail and intelligent understanding of the underlying theory and a genuine interest in the welfare of the patient will certainly lead in capable hands and minds to the production of what I have been writing about in the past 20 minutes, the making of really good microscopic tissue preparations.

NEWS AND ANNOUNCEMENTS

NOTICE TO REGISTERED MEDICAL TECHNOLOGISTS HOLDING CERTIFICATES FROM THE REGISTRY OF MEDICAL TECHNOLOGISTS OF THE AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

In order to differentiate the original M.T.'s who hold a certificate of registration from the Registry of Medical Technologists of the American Society of Clinical Pathologists from those who affix the same letters to their names and are thus trying to confuse the public, it is proposed that all those medical technologists who are identified with the Registry be urged to write, after their M.T., A. S. C. P. in parenthesis, thus, M.T. (A. S. C. P.), which clearly indicates that they are recognized by the American Society of Clinical Pathologists.

Registered medical technologists (A. S. C. P.) are entitled to protection from unwarranted intrusion of a group of technicians whom we think are not ethically entitled to the designation (M.T.) which by common usage and consent has been the property of the American Society of Clinical Pathologists for more than ten years.

COMMITTEES OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS FOR FISCAL YEAR, 1940-41

Scientific Exhibits—Marian Baker, Belleville, N. J., Chairman; Rose Matthaei, Houston, Texas; Annette Callan, Southern Pines, N. C.; Theodore Keiper, Tucson, Arizona; Harry Macko, Cleveland, Ohio; Phyllis Stanley, Newark, N. J.

Sisters' Reservations—Sister M. Eulalia, St. John's Hospital, Cleveland, Ohio, Chairman; Sister M. Inez, Mercy Hospital, Canton, Ohio; Sister M. Teresita, Coleman, Providence Hospital, Beaver Falls, Pa.

Local Arrangements—Martha Klein, Akron, Ohio, Chairman;

Harry Macko, E. Cleveland, Ohio; Kathryn Teeple, Akron, Ohio; Emily G. Smith, Lorain, Ohio.

Program—Dorothea Zoll, Philadelphia, Pa., Chairman; Frieda Claussen, St. Paul, Minn.; Evelyn Jardine, Hanover, N. H.; Rachael Lehman, Evansville, Ind.

Entertainment—Mary B. Leisman, Louisville, Ky., Chairman; Zana Skidmore, Tulsa, Okla.; Anna Mary Falck, Lancaster, Pa.; Gladys Jacobs, Bay City, Mich.; Cecelia Kortuem, Chicago, Ill.

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Credentials—Lawrence Ray, Orlando, Fla., Chairman; Hermine Tate, Lafayette, La.; John H. Conlin, Detroit, Mich.

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TO MEMBERS OF THE AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

The Nominating Committee appreciates its responsibility in selecting the slate of officers for the year 1941-1942. In order that this slate may be truly representative of the membership of the society, we wish to ask the cooperation of the membership in bringing to the attention of the committee the names of persons to be considered for office.

We shall appreciate any suggestions you might make in this

respect. Please send us the name (or names), with the qualifications for office of each person suggested (the part he has played in professional organizations, etc.), of those whom you feel would serve the society best as an officer.

Please send in your suggestions before March 1, so that the committee may have plenty of time to consider them.

Each and every suggestion will be carefully considered by the committee, and we shall endeavor to present to you at the June convention, in Cleveland, a slate of names worthy of representing the American Society.

Rose Matthaei, M.T., Chairman,
2119 Arbor Ave., Houston, Texas.
Ann Snow, M.T.,
216 East Ave. D, Park Hill,
N. Little Rock, Ark.
Rowena Johnson, M.T.,
202 Medical Arts Bldg.,
Tulsa, Oklahoma.

Nominating Committee,
American Society of Medical
Technologists.

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

Southwestern Division

Twenty-first Annual Meeting—Lubbock, Texas, April 28, 29, 30, May 1, 1941

A cordial invitation to the Medical Technologists of the southwestern portion of the United States to attend and take part in the annual meeting of the Southwestern Division of the American Association for the Advancement of Science, which will be held in Lubbock, Texas, April 28, 29, 30 and May 1, 1941. Texas Technological College is acting as the host institution.

As you perhaps know, it is not necessary to be a member of the American Association for the Advancement of Science to attend the meetings of the Southwestern Division, and present papers at these meetings. Should one be unable to attend the meetings, but have papers ready for presentation, copies of the manuscripts should be sent either to the chairman or secretary of the Biological Sciences Section, not later than five weeks prior to the meeting date. They will be presented by the officer in charge, or by anyone designated by the author.

Blanks for sending in titles, and abstracts of papers, as well as other information may be obtained by writing directly to the Sectional officers.

TO MALE ENROLLEES INTERESTED IN VOLUNTARY INDUCTION FOR ONE YEAR OF MILITARY TRAINING

The enrollment of medical technologists being conducted by the Red Cross is to provide a list of eligible technicians for service in the event of a war emergency.

As stated in a former announcement, if you are called up for service under the Selective Service and Training Act, it is expected that you will be classified for duty in the Medical Department.

Many inquiries have been received, however, from male enrollees who registered under the above Act and who are interested in rendering one year of service in the Army immediately, in the capacity for which they are enrolled, without waiting to be called up for service.

There is a basic difference between voluntary *enlistment* and voluntary *induction*. Regular enlistment in the Army is for a three-year period, whereas local Draft Boards may arrange for the voluntary induction of men desiring to take their one year of military training in advance of the normal time of being called.

It is possible for medical technologists enrolled with the Red Cross, who are desirous of taking one year of training immediately, to make the necessary arrangements for voluntary induction *through*

their local Draft Boards. At the time of induction they should display their Red Cross enrollment card to the military authorities and, *insofar as vacancies are available,* they will be classified for duty in the Medical Department in their particular technical specialty. *However, as pointed out by the Medical Department, the number of such Red Cross enrollees whose services can be utilized under the Selective Service and Training Act is limited to a smaller number than would be required in actual war. Consequently too many applications by Red Cross enrollees for advance induction will result in the Medical Department being unable to utilize the services of all.*

Under the Selective Service and Training Act all men who are inducted must serve four months as a private. At the end of that period, *insofar as vacancies are available,* Red Cross enrollees serving in the Medical Department will then be advanced to the noncommissioned status provided for their particular technical specialty.

Therefore, male enrollees between the ages of 21 to 36 have four alternatives:

1. If not called up for service under the Selective Service and Training Act, they will not be expected to render any service as a Red Cross enrollee, except when a war emergency exists or when war is imminent, which is the original basic purpose for which the enrollment is being conducted.

2. They can wait to be called for military service in accordance with the above Act, and be classified for duty in the Medical Department at that time, as outlined in a previous communication.

3. They may arrange with their local Draft Boards for *voluntary induction* for one year of training, in advance of being called in accordance with their order number as drawn in the national lottery, and be classified for duty in the Medical Department if vacancies exist. Such action, however, is subject to the conditions set forth in the 5th paragraph of this letter.

4. They may enlist in the Medical Department of the regular Army as a private for three years for general service only by applying to their nearest Army recruiting station and be promoted to non-

commissioned status when eligible in accordance with regular military procedure.

AMERICAN RED CROSS,

National Headquarters,
Washington, D. C.

Illinois

The annual meeting of the Illinois Society of Clinical Laboratory Technicians was held in Peoria, Illinois, on November 8 and 9, 1940. The following program was presented Friday, November 8, at the University Club of Peoria. Dinner meeting at 6:30 p. m.

Speaker for the occasion was Dr. William F. Petersen, Professor of Pathology, University of Illinois College of Medicine, whose subject was "Why Abraham Lincoln Felt Bad at the Meeting of Political Technicians in Peoria, Eighty Years Ago."

Saturday, November 9, Morning—Proctor Hospital, Peoria, Illinois. Business meeting. "Dioxane Method for Tissue Preparation," Dorothy Shaw, Chief Technician, Methodist Hospital, Peoria, Illinois. "Spinal Fluid Examination," Dr. L. Z. Gordon, Pathologist, Peoria State Hospital, Bartonville, Illinois. "Liver Function Tests," Dr. A. R. K. Matthews, Pathologist, St. Anthony's Hospital, Rockford, Illinois.

Saturday, November 9, Afternoon—Methodist Hospital, Peoria, Illinois. "Effect of X-Radiation on the Blood," Dr. Fred Decker, Radiologist, Methodist Hospital, Peoria, Illinois. "Round Table Discussion on Sputum and Pleural Fluid, Dr. S. A. Levinson, Pathologist and Director of Laboratories, Research and Educational Hospital, Chicago, Illinois, senior author of Levinson and MacFate "Clinical Laboratory Diagnosis."

Oklahoma

The annual meeting of the Oklahoma Society of Medical Technologists was held Saturday, November 9, 1940, at the University of Oklahoma School of Medicine, Oklahoma City. The following program was presented:

Demonstration and discussion of the Asheim-Zondek reaction and reading of vaginal smears. Lantern Slides. Demonstrated by Dr. A. A. Hellbaum, Associate Professor of Physiology.

Demonstration: The Frozen Section Method—Methods of Overcoming Objections and Difficulties—Dr. L. A. Turley, Professor of Pathology, assisted by Miss Katherine Aherhold, M.T., and Doris McLean.

Registration.

Determination of Blood Gases by the Use of the Van Slyke Apparatus. Practical Suggestions on Colorimetric Methods—Dr. I. S. Danielson, Assistant Professor of Biochemistry, assisted by Vernal Johnson, M.T., University Hospital.

Hematological Technique—Dr. J. M. Thuringer, Professor of Histology, assisted by Miss Ellen Wright, M.T., and Dorothy Nolan Brown, M.T.

"Animal Toxins"—Speech with lantern slides—Dr. A. I. Ortenburger, Professor of Zoology, University of Oklahoma, Norman.

Color Reactions Used for the Detection of Alkaloids—Demonstration by Dr. H. A. Shoemaker, Professor of Pharmacology.

Demonstration: Blood Cultures. Stool Cultures for Dysentery and Typhoid, with demonstration of special media for stool cultures. Dr. H. D. Moor, Professor of Bacteriology, assisted by Ida Lucille Wallace, M.T.

Informal dinner—Skirvin Hotel.

"Serological Tests in Virus Diseases"—Dr. K. Starr Chester, Oklahoma A. and M. College, Stillwater, Oklahoma.

Pennsylvania

The annual banquet of the Pennsylvania Society of Medical Technologists and Laboratory Technicians was held at Whitmans, 17th and Chestnut Streets, Philadelphia, November 11, 1940. There were 53 present. A very enjoyable program was prepared. Words

of greeting were extended by our president, Fannie DeSilver. Dr. Riemann of the Lankenau Hospital was toastmaster. The speakers of the evening were Dr. Tuft, from Temple University Hospital, and Dr. Engle, from the Lankenau Hospital.

Texas

The Texas Society of Medical Technologists held its eighth annual convention in San Antonio, October 11-12.

The program was very interesting; sections of the program were devoted to basal metabolisms, hematology, biochemistry, body fluids, bacteriology, and serology with papers given by members of the society. Guest speakers included Dr. Boen Swinny, San Antonio; Dr. John Chapman, San Angelo; and Dr. I. V. Irons, State Health Department, Austin, Texas.

The society was fortunate in having two of the three members of the Advisory Board present during the entire convention. They were Dr. J. J. Andujar of Ft. Worth and Dr. W. W. Waite of El Paso.

Ft. Worth was chosen as the 1941 Convention City, and the time has been changed from October to May. The meeting will precede the Texas Medical Society.

The following officers were elected for the ensuing year: President, Nylah Tom, Austin; president elect, Claryce Pitts, Austin; vice president, Will Anne Staude, Ft. Worth; secretary, Vera Dunn, Corpus Christi; treasurer, Louise Cox, Dallas; board of directors, Paul Lea, Lubbock; C. C. Hays, Waco; Pauline Dimmitt, Sherman; J. E. Storey, Abilene; H. A. Bardwell, San Antonio; Geo. Thomas, Beaumont.

Ruth Guy, Abilene, was appointed by the President, Nylah Tom, to fill the unexpired term of Vera Dunn who resigned as secretary.

Wisconsin

The fifth annual convention of the Wisconsin Association of Medical Technologists was held in Milwaukee on Saturday and Sunday, October 12 and 13, 1940. Nearly 100 attended.

The educational part of the program consisted of lectures by Dr. Thomas J. Snodgrass, Janesville, on *Sulfanilamide and Its Use in Surgery;* Dr. Merlyn C. Lindert, Milwaukee, on *Chemotherapy in the Pneumonias and Other Infectious Diseases;* Dr. Norbert Enzer, Milwaukee, on the *Participation of the Medical Technologist in the Practice of Medicine;* and Dr. David O. Holman, Milwaukee, on *Infectious Mononucleosis.*

There were also motion pictures on *Anemias* and *Thorocoplasty.* There were a number of demonstrations and discussions of laboratory methods presented by the medical technologists, including methods for sulfanilamide determination, estimation of prothrombin time (Quick and bedside methods), use of the Winthrobe hematocrit, the test for Cevitamic acid, the use of the photelometer, the determination of pH in the urine, blood cultures, and a demonstration of the new Jones waterless machine for B M R.

At the business meeting, a new constitution was adopted. The following officers were elected:

President, Louise Mead, Milwaukee; President-elect, Alice Thorngate, Madison; Secretary, Elizabeth Kullman, Milwaukee; Treasurer, Geralden Miller, Milwaukee; Historian, Laura Bates, Madison; Sergeant-at-Arms, Leila Carson, Milwaukee; Directors — Fourth District, Elaine Beck, Marshfield; Sixth District, Sr. M. Corona Rohlik, La-Crosse.